chapter

6 Smallpox and Vaccinia

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Smallpox is now a disease of historical interest only, its eradication having been certified by the World Health Assembly on May 8, 1980.[1] An exanthematous viral disease, it was once prevalent throughout the world, existing as an endemic infection wherever concentrations of population were sufficient to sustain transmission. Outbreaks of variola major, the only known variety until the end of the 19th century, resulted in case-fatality rates of 20% or more. Most of those who survived had distinctive residual facial pockmarks, and some were blind. A second variety, variola minor, produced less severe illness and was associated with case-fatality rates of 1% or less. It was first described in South Africa by de Korte[2] and in the United States by Chapin[3] and subsequently became the prevalent variety throughout the United States, parts of South America, and Europe as well as some areas of eastern and southern Africa.[4]

Because there was no animal reservoir of smallpox and no human carriers, the virus had to spread continually from human to human to survive. Thus, historians speculate that it must have emerged sometime after the first agricultural settlements, about 10,000 BC.[5] The first certain evidence of smallpox in the ancient world comes from mummified remains of the 18th Egyptian dynasty (1580 to 1350 BC) and of the better known Ramses V (1157 BC).[6] Written descriptions of the disease, however, did not appear until the 4th century AD in China[7] and the 10th century in southwestern Asia.[8]

From northeastern Africa, smallpox was probably carried by Egyptian traders to India during the first millennium BC,[4] where it became established as an endemic infection. Whether smallpox persisted in Africa is uncertain. Although epidemics of disease are described in the Bible and in Greek and Roman literature, descriptions of clinical signs are sparse. Only one of these epidemics can be identified with some certainty as smallpox.[7] It occurred in Athens beginning in 430 BC and is described by Thucydides. There is, however, no original Greek or Latin word for smallpox despite its distinctive rash.[9] From the populated endemic areas of Asia and perhaps Africa, smallpox spread with increasing frequency into less populous areas of these continents and into Europe, becoming established as an endemic infection when populations increased sufficiently in number.

The name *variola* was first used during the 6th century by Bishop Marius of Avenches (Switzerland), the word being derived from the Latin *varius* (spotted) or *varus* (pimple).[10] Although Marius provides no clinical description of the disease concerned, there is little doubt that smallpox had already become endemic in some areas of Europe by this time.[7] In the Anglo-Saxon world, by the 10th century, the word *poc* or *pocca*, a bag or pouch, described an exanthematous disease, possibly smallpox, and English accounts began to use the word *pockes*. With the appearance of syphilis in Europe in the late 15th century, writers began to use the prefix *small* to distinguish variola, the smallpox, from syphilis, the great pox.[11]

In the early 16th century, smallpox began to be imported into the Western Hemisphere. Catastrophic epidemics followed, which literally decimated Amerindian tribes and resulted in the collapse of both the Aztec and Incan empires.[5] Central and southern Africa probably became endemic for smallpox about this time or soon thereafter.

The impact of smallpox on history and human affairs was profound.[7] Deities to smallpox became a part of the cultures of India, China, and parts of Africa. In Europe, as of the end of the 18th century, an estimated 400,000 persons died annually from smallpox, and survivors accounted for one third of all cases of blindness. During the 18th century alone, five reigning European monarchs died of smallpox, and the Austrian Hapsburg line of succession shifted four times in four generations.

A method for protection against naturally acquired smallpox infection appears to have been discovered in India sometime before AD 1000.[12, 13] There it became the practice to deliberately inoculate, either into the skin or by nasal insufflation, scabs or pustular material from lesions of patients. This practice resulted in an infection that was usually less severe than an infection acquired naturally by inhalation of droplets. From India, the practice spread to China, western Asia, and Africa and finally, in the early 18th century, to Europe and North America.[14] Case-fatality rates associated with variolation, as it was called, were about one tenth as great as when infection was naturally acquired, but those infected in this manner were capable of transmitting smallpox by

Figure 6–1. Edward Jenner (1749–1823) demonstrated that a person inoculated and infected with cowpox was protected against smallpox. The procedure, which he called vaccination, represented the first use of a vaccine in the prevention of disease. (Courtesy of the Institute of the History of Medicine, The Johns Hopkins University, Baltimore, MD.)

droplet inhalation to others. After cowpox began to be used as a protective vaccine, the practice of variolation diminished. Even as recently as the 1960s and 1970s, however, variolation continued to be performed among remote populations in some parts of Ethiopia, western Africa, Afghanistan, and Pakistan.[4]

In 1796, Edward Jenner (Fig. 6–1) demonstrated that material could be taken from a human pustular lesion caused by cowpox virus (i.e., an orthopoxvirus closely related to variola virus) and inoculated into the skin of another person, producing a similar infection.[15] He showed that the individual was protected from inoculation with smallpox after recovery. He called the material *vaccine*, from the Latin *vacca*, meaning cow, and the process *vaccination*. Pasteur,[16] in recognition of Jenner's discovery, later broadened the term to denote preventive inoculation with other agents. Jenner's discovery, one of the most important in medical history, was immediately recognized for its significance. Within 5 years, his paper had been translated into six other languages,[17] and the vaccine had begun to be employed widely in many countries of Europe; within a decade, it had been transported to countries throughout the world. The chronicles of the de Balmis expedition of 1803 to 1806 vividly describe the transport of the vaccine by sea to Spanish colonies in the Americas and Asia by arm-to-arm vaccination of orphaned children.[18, 19]

As the 19th century progressed, however, the initial wave of enthusiasm for vaccination subsided when difficulties were experienced in sustaining the virus through arm-to-arm inoculation and when it was found that, on some occasions, syphilis was transmitted in the process.[20, 21] Although vaccination material, dried on threads or ivory points, could be transported over long distances, it was often found, on receipt, to be noninfectious. When fresh material was sought, problems occurred in finding cows or horses with infections caused by cowpox or a related orthopoxvirus.[22] In some areas, significant opposition occurred among religious leaders and anti-vaccinationist societies who opposed the principle of infecting humans with an animal disease.[23] Confidence in the procedure was also diminished by the occurrence of smallpox in some who had previously been successfully vaccinated. Jenner had forcefully contended that protection was lifelong, as was the case after natural smallpox, but it soon became apparent that this was not so. Although the need for revaccination was demonstrated early in the century,[24] this practice was not widely accepted until many decades later.

Growth of the virus on the flank of a calf offered the prospect for provision of an adequate and safer supply of vaccine material. Although this approach was employed in Italy as early as 1805,[25] it appears to have been unknown elsewhere until it was more widely publicized at a medical congress in 1864.[26] Thereafter, the practice was gradually adopted in other countries, although arm-to-arm vaccination in England, for example, continued until it was finally banned in 1898.[27] With an ensured source of vaccinia, the numbers of vaccinations in Europe increased, and the incidence of smallpox in the more industrialized countries diminished more rapidly. Not until after World War I, however, did most of Europe become smallpox free, and not until after World War II was transmission stopped throughout Europe and North America.

In most other parts of the world, especially in tropical and semitropical areas and in the less developed countries, smallpox continued largely unabated until the middle of the 20th century. In these countries, continuing difficulties were experienced in sustaining the virus through arm-to-arm inoculation. After calves began to be used for vaccine production, the harvested vaccine remained viable for only 1 or 2 days at ambient temperatures, thus limiting its widespread application. The only control programs that were notably successful were those in Indonesia and in certain of the French colonies, which, in the 1920s, began using a specially prepared and more stable air-dried[28] or freeze-dried[29] vaccine.

In the late 1940s, a commercially feasible process for large-scale production of a stable freeze-dried vaccine was perfected by Collier.[30] This process offered vastly better possibilities for smallpox control. Recognizing the value of such a vaccine, the Pan American Sanitary Organization[31] decided, in 1950, to undertake a hemisphere-wide eradication program and by 1967 succeeded in eliminating smallpox from all countries of the Americas except Brazil. Meanwhile, in 1958, the Union of Soviet Socialist Republics proposed to the World Health Assembly that a global smallpox eradication program be undertaken,[32] and this was so decided the following year.[33] Some progress was made during the period from 1959 to 1966, but the results overall were disappointing. Finally, in 1966, the World Health Assembly decided to intensify the eradication program by providing additional funds specifically for this effort.[34]

During 1967, the year the Intensified Global Eradica-

tion Program began, an estimated 10 to 15 million smallpox cases[1] occurred in 31 countries in which the disease was endemic. The campaign was based on a twofold strategy: (1) mass vaccination campaigns in each country, using vaccine of ensured potency and stability that would reach at least 80% of the population and that would be assessed by independent teams, and (2) development of a system to detect and contain cases and outbreaks.[35] Numerous problems had to be surmounted, including deficient supervision and discipline in national health services, epidemic smallpox among refugees fleeing areas stricken by civil war and famine, shortages of funds and vaccine, and a host of other problems posed by difficult terrain, climate, and cultural beliefs.[36–38] Despite the problems, steady progress was made, and on October 26, 1977, the last known naturally occurring case of smallpox was recorded in Merka, Somalia.[39] Two further cases occurred in 1978 as a result of a laboratory infection in Birmingham, England,[40] but these cases were the last. Detailed accounts of national programs are provided in books dealing with those in India,[41, 42] Bangladesh,[43] Ethiopia,[44] and Somalia.[45]

An extensively illustrated volume entitled *Smallpox and Its Eradication*[4] provides a detailed account of the eradication campaign as well as an overall account of progress in smallpox control throughout history. It also gives a description of the virology, the clinical features, and the pathogenesis of the disease. Complementing this text is a historical record of smallpox, *Princes and Peasants*, by Hopkins.[7]

BACKGROUND

Clinical Description

Smallpox had an incubation period of about 12 days, with a range of 7 to 17 days. A 2- to 5-day period of high fever, malaise, and prostration with headache and backache was followed by the development of a maculopapular rash. The rash appeared first on the mucosa of the mouth and pharynx, the face, and the forearms and spread to the trunk and legs. Within 1 to 2 days, the rash became vesicular and then pustular. The pustules were characteristically round, tense, and deeply embedded in the dermis; crusts began to form about the eighth or ninth day. When they separated, they left pigment-free skin and, eventually, pitted scars. The eruption was characteristically more extensive on the face and distal parts of the arms and legs (Fig. 6–2), and lesions were occasionally found on the palms and soles. Death, when it occurred, was usually late in the first week or during the second week of the illness and was commonly due to the effects of an overwhelming viremia. On occasion, a severe and always fatal hemorrhagic form occurred, with extensive bleeding into the skin and gastrointestinal tract, followed by death within a few days.

Illness caused by variola major was generally more severe, with a more extensive rash, a higher fever, and a greater degree of prostration, than illness caused by variola minor. A milder form of disease was also seen among those who had previously been vaccinated; the rash in such persons tended to be more scant and atypical and the evolution of lesions more rapid.

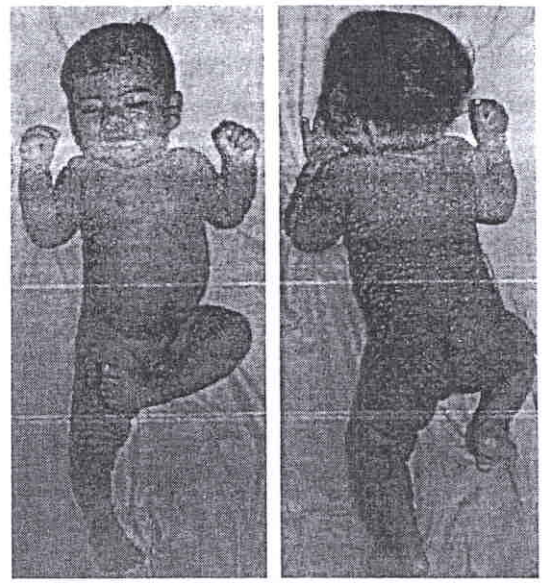

Figure 6–2. A typical case of variola major about 7 days after the onset of rash. (World Health Organization Smallpox Recognition Card.)

Cases of smallpox among pregnant women often resulted in spontaneous abortion of the fetus or a stillborn infant with evidence of lesions on the skin.

Virology

Variola virus belongs to the genus *Orthopoxvirus*, family Poxviridae, which includes the agents of vaccinia, monkeypox, cowpox, camelpox, and ectromelia.[46] All species exhibit extensive serological cross-reactivity, both in in vitro tests and in experimental animals. The poxvirus genome, the largest of all virions, is a brick-shaped structure with a diameter of about 200 nm, consisting of a single molecule of a double-stranded DNA. It differs from most other DNA viruses in that it multiplies in the cytoplasm rather than in the nucleus of susceptible cells.

The orthopoxviruses grow and produce a cytoplasmic effect in cultured cells derived from many species,[47, 48] although they generally grow best in cells from humans and other primates. The four that infect humans (variola, vaccinia, cowpox, and monkeypox viruses), however, cannot be differentiated readily from one another in most cell cultures. For diagnostic purposes, therefore, they are customarily grown on the chorioallantoic membrane of 10- to 12-day-old chick embryos on which they produce pocks characteristic of their species.[49]

Pathogenesis

Natural smallpox infection occurred by implantation of variola virus on the oropharyngeal or respiratory mucosa. Virions in droplets expressed from nasal and oropharyngeal secretions were far more infectious than

those bound in the fibrin mesh of scabs. After migration to and multiplication in regional lymph nodes, an asymptomatic viremia developed about the third or fourth day, followed by multiplication of virus in the spleen, bone marrow, and lymph nodes. A secondary viremia began about the eighth day, accompanied by fever and toxemia. The virus, contained in leukocytes, then localized in small blood vessels of the dermis and beneath the oral and pharyngeal mucosa and subsequently infected adjacent cells. In the skin, this process resulted in the characteristic maculopapular lesions and, later, the vesicular and pustular lesions, which, for reasons unknown, were more extensive on the face and distal extremities. Lesions in the mouth and pharynx ulcerated quickly because of the absence of a stratum corneum, releasing large amounts of virus into the saliva about the time the cutaneous rash first became visible. Virus titers in saliva were highest during the first week of illness, corresponding with the period during which patients were most infectious.

Hemagglutinin-inhibiting (HI) and neutralizing antibodies could be detected beginning about the sixth day of illness, or about 18 days after infection, and complement-fixing (CF) antibodies approximately 2 days later.[50, 51] Neutralizing antibodies were long lasting, whereas HI antibodies declined to low levels within 5 years, and CF antibodies rarely persisted for longer than 6 months. Little is known about the development of cell-mediated immunity.

Vaccinia-induced antibody responses were more rapid. They could be detected as early as the 10th day[52] after primary vaccination and within a week of revaccination. This accelerated response was associated with complete or partial protection of persons vaccinated at or soon after exposure.

Except for the lesions in the skin and mucous membranes and reticulum cell hyperplasia, other organs were seldom involved in variola infection. Secondary bacterial infection was not common, and death, when it occurred, probably resulted from the toxemia associated with circulating immune complexes and soluble variola antigens.[53] Encephalitis sometimes ensued that was indistinguishable from the acute perivascular demyelination observed as a complication of infection due to vaccinia, measles, and varicella.

As the patient recovered, the scabs separated and the characteristic pitted scarring gradually developed (Fig. 6–3). The scars were most evident on the face and resulted from the destruction of sebaceous glands followed by shrinking of granulation tissue and fibrosis.

Diagnosis

Most cases of smallpox were able to be diagnosed readily by the appearance of the typical deep-seated rash, the centrifugal distribution of lesions, and the fact that all lesions were at the same stage of development on any given area of the body. The infrequent hemorrhagic cases were often initially misdiagnosed as meningococcemia, acute leukemia, or drug toxicity, but their identity was soon established by examination of other

Figure 6–3. An Afghani boy with characteristic residual facial scars after smallpox. (Courtesy of the World Health Organization, Geneva, Switzerland.)

patients who were the source of infection or to whom disease had been transmitted. Varicella was by far the most frequent disease to be confused with smallpox. Smallpox patients who had previously been vaccinated and those with variola minor sometimes exhibited a sparse and sometimes atypical rash with minimal systemic symptoms that resembled varicella; severe cases of varicella in adults with extensive rash were also sometimes mistaken for smallpox.[54]

Diagnosis of a poxvirus infection can be rapidly confirmed by electron microscopic identification of virus particles in vesicular or pustular fluid or scabs. Differentiation as to which orthopoxvirus is the responsible agent is usually apparent from its characteristics of growth on the chorioallantoic membrane of chick embryos, although confirmation by other biological tests is sometimes necessary.

Recovered patients exhibit high titers of neutralizing, HI, and CF orthopoxvirus antibodies, but cross-absorption studies are required to identify which of the orthopoxvirus species is the agent responsible for illness. Characteristic residual facial scars are most useful in documenting prior cases of variola major,[55] but such scars were too infrequent to be of value in identifying recovered cases of variola minor.[56]

EPIDEMIOLOGY

Transmission

Transmission of variola virus, with few exceptions, resulted from droplets expressed by a patient from the oral, nasal, or pharyngeal mucosa that were inhaled by susceptible persons in close contact with the patient. Such transmission was possible from the time of onset

of rash and was most frequent during the first week of the exanthem. Virus was also present in high titer in scabs that had separated from the skin lesions.[57] Epidemiological evidence showed that infected scabs played a negligible role in transmission of infection, presumably because the virus was tightly bound in its fibrin matrix. It was standard practice, nevertheless, during the global eradication program, to isolate patients until all scabs had separated from the skin. Airborne infection over longer distances was uncommon, although two outbreaks within hospitals demonstrated this to be possible.[58,59] The infection of persons such as laundry workers who handled linen from patients has also been repeatedly documented.[9] However, various older accounts that purport to document transmission over great distances on other fomites, such as carpets, letters, and cotton rags, are suspect because the virus does not survive for long periods at customary ambient temperatures.[60]

Another method of transmission, the ancient practice of variolation (inoculation into the skin of material from pustules or scabs from patients), continued in a number of remote areas until August 1976 and was responsible for many cases in Afghanistan and Ethiopia. Those individuals so inoculated often developed extensive rash and transmitted infection to susceptible contacts by droplet infection.

Geographical Scope and Epidemiological Characteristics

Smallpox was once worldwide in scope, persisting as an endemic disease in areas where susceptible populations were sufficiently large to permit year-round transmission. In more remote or isolated areas, epidemics occurred when the disease was introduced, but because infection resulted in essentially permanent immunity, transmission eventually ceased when the number of susceptible contacts diminished to low numbers. Before vaccination was practiced, almost everyone eventually contracted the disease.

When a vaccine became available, its introduction followed a common pattern, at first being most extensively used among middle- and upper-income groups in or near cities where the vaccine was produced and in more prosperous countries. Thus, during recent years, smallpox incidence was highest among lower socioeconomic groups in urban areas and in the rural areas of developing countries.

The seasonal occurrence of smallpox was similar to that of varicella and measles, its incidence being highest during winter and spring. This factor was consonant with the observation that the duration of survival of the virus in the aerosolized form was inversely proportional to both temperature and humidity.[61] Such seasonal variation was undoubtedly amplified in many countries by social events, such as the congregation of large numbers of people during the dry season at festivals and marriage parties, and the movement of nomads during this period. Where there was less variation in temperature and humidity, as in equatorial areas of Indonesia and Zaire (now the Democratic Republic of Congo), there was little discernible fluctuation in incidence throughout the year.

There were also longer term trends in incidence in the endemic areas, which resulted in major epidemics at intervals of 4 to 7 years,[4,62] presumably relating to an accumulation of susceptible persons and in part consequent to events, such as famine and civil war, that resulted in extensive refugee movements and widespread dissemination of the virus.

Within the household, smallpox was as infectious as chickenpox but less infectious than measles.[63–65] With few exceptions, however, smallpox spread less widely and rapidly than these diseases. This finding can be accounted for by the fact that transmission of variola virus did not occur until onset of rash, as attested by numerous epidemiological observations. By then, most patients were already confined to bed because of the high fever and malaise of the prodromal illness; secondary cases were usually restricted to the few who came in contact with them in the household or hospital. On average, a given case of smallpox seldom resulted in more than two to five cases in a subsequent generation, most of whom were relatives or friends. For this reason, smallpox outbreaks tended to be clustered in a segment of a town or village and in localized areas of a province or district.[66–69] Most outbreaks, therefore, could be contained successfully by vaccination of a comparatively small number of residents in and near the houses in which patients lived.

The age distribution of smallpox cases depended on the acquired immunity of the population, whether by vaccination or by infection. Cases among adults were regularly found, however, even as recently as 1974 to 1975 in India, where vaccination had been widely practiced and smallpox was endemic (Table 6–1). During this period, 21% of a carefully documented series of 23,546 patients were older than 20 years, and 2% or 412 of these patients were older than 50 years. In western Africa during the 1967 to 1969 period, most cases were in rural villages, and the age distribution of cases approximated the age profile of the population.[70] In all countries, males and females were equally affected.

Where the Asian form of variola major was prevalent, case-fatality rates were about 20% overall, but for those younger than 1 year, they ranged from 40 to 50%.

Table 6–1. **INDIA: CASES OF SMALLPOX, DEATHS, AND CASE-FATALITY RATES, BY AGE GROUP, 1974 TO 1975**

AGE GROUP (yr)	NUMBER OF CASES (% DISTRIBUTION BY AGE)	NUMBER OF DEATHS	CASE-FATALITY RATE (%)
<1	1373(6)	597	43.5
1–4	5867(25)	1436	24.5
5–9	5875(25)	783	13.3
10–19	5542(23)	432	7.8
≥20	4889(21)	855	17.5
Total	23,546(100)	4103	17.4

From Basu RN, Jesek Z, Ward NA. The eradication of smallpox from India. In History of International Public Health No. 2. New Delhi, World Health Organization, South-East Asia Regional Office, 1979, p 59.

Variola major in Africa was a somewhat milder disease, with age-standardized, case-fatality rates 20 to 30% lower. Variola minor, which after 1967 was present only in Brazil and southern and eastern Africa, resulted in case-fatality rates of 1% or less.

The Significance of Smallpox as a Public Health Problem

During recent centuries, smallpox was the most universally feared of all diseases. It could occur and spread in any country, and case-fatality rates were little altered by therapy. It was not dependent on a vector; thus, in contrast to malaria or yellow fever, it could occur anywhere in any season. Better sanitation and improved economic conditions diminished the concern for diseases such as cholera and typhoid, but such measures had little influence on smallpox.

Jenner's discovery of a protective inoculation was understandably lauded, and although it conferred a high level of protection, periodic revaccination was necessary. No country was able to sustain a vaccination program that ensured that everyone in the population was fully protected at all times; thus, all countries feared possible smallpox importations and subsequent spread. For this reason, through the mid-1970s, all countries required travelers to present certificates attesting to the fact that they had been vaccinated within the preceding 3 years. Even those countries that were smallpox free continued national vaccination programs in the belief that this practice would serve to impede the spread of disease, if it were introduced. When importations occurred, they were frequently accompanied by public hysteria and a demand for mass vaccination.

The costs of preventive measures for smallpox were substantial. Sencer and Axnick[71] documented activities and expenditures for smallpox control in the United States during 1968, nearly 20 years after its last case of smallpox. In all, nearly 15 million persons were vaccinated that year, and because of vaccine complications, 240 required hospitalization, 9 died, and 4 were permanently disabled. The total costs to the country, including the costs of quarantine services, were estimated to be $150 million. Other countries, such as the United Kingdom and the Federal Republic of Germany, maintained special buildings to be opened for the hospitalization of patients when imported cases of smallpox occurred. When importations occurred, extreme measures were frequently taken, such as in Yugoslavia in 1972 when the entire population was vaccinated, borders were closed to commerce, and thousands who had possibly been exposed were isolated in hotels coopted for this purpose.[72]

Although the concern was great, importations of smallpox into industrialized Europe, North America, and Japan were relatively infrequent after 1958. There was a total of only 36 episodes, with none after 1973.[4] These episodes resulted in 574 cases and 90 deaths, more than half being the result of exposure to patients in hospitals.[73] Most importations resulted from improperly vaccinated visitors returning from Bangladesh, India, and Pakistan, although importations from Africa and South America were also documented.

Countries in the endemic regions of the world experienced more frequent importations because of travelers and nomads moving freely across long open borders and serving to reinfect persons in countries that had become smallpox free. Relative to the extent and numbers of travelers, however, importations were comparatively few. This reflected the fact that smallpox outbreaks tended to remain localized, usually spread by relatives or friends to adjacent houses or villages in an area. Those who traveled were usually adults who were immune from smallpox as a result of past infections or immunizations; those who traveled long distances by plane tended to be fairly affluent and thus better vaccinated and with less contact with the lower socioeconomic groups and rural peoples, among whom most cases occurred.

ACTIVE IMMUNIZATION

Vaccine Strains

Strains of Vaccine and Their Passage. Many strains of vaccinia, known by different names, have been used by different producers during this and the past century, but little is known about their origins or passage histories. Characterization of strains is further complicated by the fact that a seed lot system for vaccine production was not used until the 1960s. Thus, even those strains with common names and ancestors have different passage histories, having been passed sequentially through a variety of vaccinifers, such as cows, sheep, and water buffalo, with periodic passages through rabbits, horses, and even humans. Indicative of the ignorance of vaccine technology until recent decades is a statement of the Ministry of Health of Great Britain, which, in 1928,[74] advised that seed lymph could be obtained from (1) "smallpox direct"; (2) cowpox; (3) horsepox, sheep-pox, or goatpox; and (4) vaccinia in the human body.

Jenner is believed to have used cowpox in vaccination, but the vaccinia virus strains used most recently are a different species of orthopoxvirus with distinctive DNA maps that are similar to each other but different from both cowpox and variola. That the vaccinia strains are not mutants of variola virus seems certain,[75] but where the present vaccinia species arose is unknown. It may have arisen either as a hybrid of cowpox and another orthopoxvirus or through thousands of serial passages under artificial conditions of culture. It is also possible that the species represents a laboratory survivor of a now naturally extinct species of orthopoxvirus.[76]

In 1958, a World Health Organization (WHO) Study Group first recommended that a seed lot system be employed in vaccine manufacture. Beginning in 1967, an increasing number of vaccine producers, encouraged by the WHO, began to use one of two strains. Most common was the Lister strain from the Lister Institute, England, which was propagated as seed virus by the National Public Health Institute of the Netherlands for distribution by the WHO. The second strain was the New York City Board of Health strain, propagated by

Wyeth Laboratories, Radnor, Pennsylvania, United States. Two of the largest countries, China and India, used other strains called, respectively, the Temple of Heaven strain and the Patwadanger strain.

During the 1930s, vaccinia strains began to be attenuated by serial passages in an effort to diminish the incidence of complications; the first was the Rivers strain, which was derived from the New York City Board of Health strain.[77] Three principal variants were developed that had been passed repeatedly through rabbit testis, chick embryo explants, and chorioallantoic membranes of embryonated hens' eggs.[78] Rivers and colleagues[79, 80] showed that the "second revived strain" produced less severe reactions in rabbits and humans than did the New York City Board of Health strain, especially if it was inoculated intradermally. This strain, administered with 2 mL of vaccinia immunoglobulin, was used for primary vaccination of 60,000 Dutch army recruits by van der Noordaa and colleagues.[81] One mild case of postvaccinal encephalitis occurred, but this was a lower incidence than that noted after administration of other strains. The resultant neutralizing antibody titers, however, were lower than those usually observed. This called into question the level of protection provided against smallpox, and the strain was not further employed.

Another variant of the Rivers vaccine, the CVI-78 strain, was also found to produce less severe local reactions, and although used to vaccinate children with eczema,[82] it was not thought likely to provide adequate protection against smallpox.[83] A large-scale comparative trial sponsored by the National Institutes of Health[84, 85] showed that the CVI-78 strain was 10-fold less infectious than the Lister strain and New York City Board of Health strains and produced smaller skin lesions and fewer febrile responses. Only 30% of children, however, exhibited neutralizing antibody, and after challenge vaccination with a standard strain, 25% still did not respond with neutralizing antibody.

Another attenuated vaccine, the modified vaccinia virus Ankara (MVA) strain produced by Stickl and collaborators[86] through passage in chick embryo fibroblast cells, had characteristics similar to those of the CVI-78 strain.[87] Some workers believed that a sequential vaccination schedule using the CVI-78 or MVA strain, followed after some months by application of a conventional strain, offered prospects for protection against smallpox with fewer complications. However, whether persons without neutralizing antibody response would be protected against natural challenge remained an unanswered question.

A more satisfactory attenuated strain, LC 16m8, was produced by Hashizume[88–90] through passage at low temperature in rabbit kidney cells. This strain produced a satisfactory immune response in humans (HI and neutralizing antibodies), and in a field trial of 50,000 persons, it was found to produce a markedly lower frequency of reactions than that noted for other strains.[91] However, the achievement of smallpox eradication precluded use of this vaccine under circumstances of natural challenge.

Dosage and Route. The vaccine is inoculated intradermally with use of a bifurcated needle. Vaccine, as

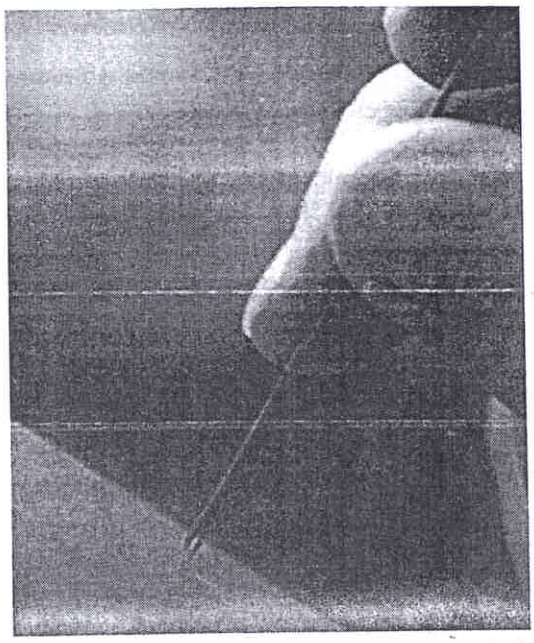

Figure 6–4. The bifurcated needle positioned to begin multiple puncture vaccination. (Courtesy of the World Health Organization, Geneva, Switzerland.)

reconstituted for use with the bifurcated needle, is required to have a titer of not less than 10^8 pock-forming units per milliliter when it is assayed on the chorioallantoic membranes of 12-day-old chick embryos. Approximately 0.0025 mL of vaccine adheres by capillarity to the tines of the needle when it is dipped into the vaccine. The needle is positioned vertically to the skin surface, usually the lateral surface of the upper arm (Fig. 6–4), and 5 to 15 rapid strokes are made. These strokes are sufficiently vigorous that within 20 to 30 seconds, a trace of blood appears at the vaccination site.

Constituents of Vaccine. Most vaccine now available for use is grown on the skin of a calf and harvested after sacrifice of the animal. The vaccine is purified by the addition of fluorocarbon and differential centrifugation, and its bacterial content is reduced by the addition of phenol. Peptone is added as a stabilizing agent, and the vaccine is freeze-dried. Because of its source, the vaccine inevitably contains some bacteria, but properly prepared, the number of bacteria is 10/mL or less. Microbiological examination must confirm that none is a human pathogen. For reconstitution of the vaccine for multiple puncture vaccination, a solution of 50% (volume per volume) glycerin in McIlvaine solution is used; for vaccine intended for jet injection, saline is used.

Laboratories in Brazil, New Zealand, Sweden, and the United States (e.g., Texas State Health Department) harvested vaccinia virus from the chorioallantoic membranes of chick embryos, a simple process that permits production of a bacteria-free vaccine. However, vaccine from this source proved difficult to produce in a satisfactory thermostabile freeze-dried form, and as far as is known, only Sweden produced the vaccine in eggs that were free of avian leukosis virus.

Vaccinia virus grown in tissue culture also proved difficult to produce as a thermostabile freeze-dried prod-

uct, but Hekker and colleagues[92] eventually achieved this result using primary rabbit kidney cells. In field trials, the vaccine was comparable to vaccine grown on calf skin,[93, 94] but because of the approaching conclusion of the smallpox eradication program, the WHO made no effort to introduce the method for use in other laboratories.

Producers. Because of the eradication of smallpox and the cessation of routine vaccination, the number of production laboratories diminished from 76 in 1977 to 11 in 1985. The few remaining laboratories are in the industrialized countries and are engaged only in the preparation of finished vaccine from bulk preparations harvested in quantity some years ago and preserved by freezing. Virus grown in tissue culture is available in the Netherlands (Lister strain) and Japan (LC 16m8 strain); other countries use vaccine grown on calf skin (primarily Lister and New York City Board of Health strains).

Storage Conditions. Freeze-dried smallpox vaccine is the most stable of currently available vaccines. The vaccine can be preserved indefinitely at −20°C and most batches are equally well preserved at 4°C. International standards require that the vaccine in its freeze-dried form maintain full potency when it is incubated at 37°C for 1 month. Studies of vaccine produced at the Lister Institute, however, demonstrated that the vaccine retained full potency for 64 weeks when it was incubated at temperatures of up to 45°C and for 104 weeks at 37°C.[95] Not all vaccines were this stable, but assay of vaccines produced in India and the former USSR and retrieved from the field revealed batches of vaccine that met potency standards after 6 to 9 months of exposure at high ambient summer temperatures. After reconstitution, the vaccine is much more sensitive both to temperature and to exposure to direct light. During the eradication program, unused reconstituted vaccine was routinely discarded at the end of each day, although it can be preserved in this form for at least 1 week at 4°C.

Results of Vaccination

Immune Response. After primary vaccination, neutralizing and HI antibodies develop about the 10th day and are present in almost all persons by the end of 2 weeks; CF antibodies develop in less than half of the vacinees.[52] Because the antibody response after primary vaccination usually occurs 4 to 8 days earlier than the response after naturally acquired smallpox infection,[96] primary vaccination even after exposure sometimes modified or aborted an overt attack of smallpox. The neutralizing antibodies are most persistent and may be detected for 20 years or more; HI and CF antibodies, however, are usually not detectable beyond 6 months. Little is known about the cell-mediated immunity that is induced, although Pincus and Flick[97] demonstrated the beginning development of delayed hypersensitivity, an index of cell-mediated immunity, as early as 2 days after vaccination. Antibody response after revaccination is more rapid, usually within 7 days, and antibody titers are generally higher. However, some persons who exhibit a substantial rise in neutralizing antibody titer after revaccination fail to exhibit a rise in either HI or CF antibody levels.

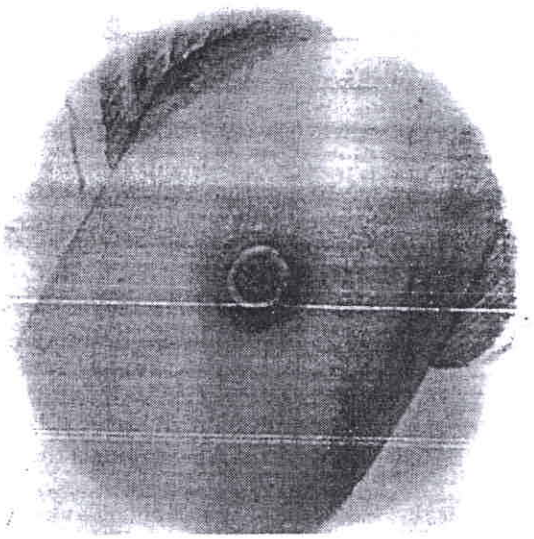

Figure 6–5. A primary vaccination response on the ninth day after inoculation shows erythema surrounding a pustular lesion. Although the picture is from a colored drawing made by Captain C. Gold in 1801, the lesion shown is indistinguishable from contemporary responses to primary vaccination. (Courtesy of the Library, London, Wellcome Institute for the History of Medicine.)

Successful primary vaccination results in virus proliferation in the basal cells of the epidermis, producing the typical jennerian vesicle (Fig. 6–5). A papule with surrounding erythema develops in 3 to 5 days, rapidly becoming a vesicle and later a pustule. It reaches its maximum size after 8 to 12 days. A scab forms that separates at 14 to 21 days, leaving a typical vaccination scar. A low-grade fever usually accompanies the development of the pustule, and swelling of the draining lymph nodes, associated with tenderness, is often observed. Viremia may occasionally occur[98] between the third and tenth days, and vaccinia virus can sometimes be isolated from tonsillar swabs.[99]

An individual's response to revaccination depends on the level of immunity. Erythema typically develops within 24 to 48 hours as a classic delayed hypersensitivity reaction. As Benenson[100] has shown, this reaction can be elicited with both live and inactivated vaccine. Persons with some residual cell-mediated immunity, but not enough to inhibit viral replication, develop erythema and sometimes a pustule at the site of a vesicle, both of which evolve in a sequence more rapid than that in a primary vaccination reaction. Those with substantial immunity may experience no more than the hypersensitivity reaction.

Because it is impossible to distinguish between a hypersensitivity reaction due to the use of impotent vaccine and a similar reaction due to a high level of immunity, the WHO Expert Committee on Smallpox[101] recommended that such a response be termed an *equivocal reaction*. For persons with equivocal reactions, repeated vaccinations were advised. Others who exhibited evidence of virus proliferation at 6 to 8 days, as manifested by a pustular lesion or an area of induration surrounding

a central lesion, were said to have experienced a *major reaction.*

Protection Afforded by Vaccination. Reliable data are surprisingly sparse as to the efficacy and durability of protection afforded by vaccination. Before 1967, when the intensified global eradication program began, revaccination every 3 to 10 years was considered essential to ensure protection. In part, this practice was based on early data largely from the United Kingdom, such as those provided by Hanna,[102] and on more recent data from India,[103] which compared the frequency of cases among those with and without vaccination scars. However, the vaccine in use in the populations studied was far lower in titer than that used after 1967; most of the vaccine was heavily contaminated with bacteria. In India, the vaccination instrument that was used (i.e., the rotary lancet) was found to produce localized sepsis and an apparent scar, even when only the diluent was applied. Estimates of protection after successful vaccination were therefore almost certainly understated in these as in other early studies. Another observation that suggested that protection might persist for no more than 3 to 5 years was the increasing proportion of persons who exhibited a major reaction to revaccination beginning about this time. Mistakenly, resistance to intradermal inoculation with vaccinia virus was equated with resistance to variola virus acquired by droplet inhalation.

From studies conducted after 1967, it became apparent that vaccinial immunity was far more durable than most investigators believed. It was found that with the available higher titer vaccines, major reactions could be induced in persons successfully vaccinated as recently as 3 to 6 months before and, indeed, in almost all of those who had experienced smallpox only 1 year previously.[104] Because natural infection effectively confers permanent immunity, it was apparent that the ability of vaccinia virus to proliferate on inoculation into the basal cells of the dermis correlated poorly with the level of protection afforded against natural infection. Moreover, in most countries, 90% or more of cases were among individuals without vaccination scars. This finding led to surveys in the endemic countries that disclosed vaccine-efficacy ratios of 80% or more among those vaccinated 20 years previously. Heiner and colleagues,[65] however, showed that this protection could not be attributed solely to the vaccine. They discovered that previously vaccinated persons often developed inapparent infection with substantial increases in antibody levels. Immunity in the endemic countries was thus a composite of past experiences with both vaccinia and variola infections. Data from countries where smallpox was introduced after an absence of many years provide insufficient information to permit calculation of vaccine-efficacy ratios, but they do indicate that the vaccine provides substantial long-term protection against a fatal outcome.[73] Among 680 cases of variola major occurring after importations of smallpox into Europe, the case-fatality rate was 52% among those who had never been vaccinated, 1.4% among those vaccinated up to 10 years before exposure, and 11.1% among those vaccinated more than 20 years before.

Simultaneous Administration with Other Antigens. It has been shown that smallpox vaccine can be administered at the same time as a number of other antigens, usually at a different site, with levels of safety and efficacy comparable to those observed when the vaccines are given separately. Simultaneous administration of oral poliovirus and smallpox vaccines became a routine practice in many countries beginning in the 1960s.[105, 106] Smallpox and bacille Calmette-Guérin (BCG) vaccines began to be administered to newborns in Hong Kong in the 1960s[107]; this became a common practice in many African countries in the late 1960s. Yellow fever and smallpox vaccines were mixed and administered successfully in many French-speaking areas of western Africa,[108] and measles and smallpox vaccines were simultaneously administered in a program throughout western Africa from 1967 to 1972.[109] Mixing of smallpox, yellow fever, and measles vaccines for inoculation by jet injection resulted in a diminished immune response to yellow fever,[110] but responses were satisfactory when different sites of inoculation were used. Ruben and colleagues[111] extended the studies to the simultaneous administration by jet injection, but at different sites, of smallpox, yellow fever, measles, and diphtheria-pertussis-tetanus (DPT) vaccines. Systemic reactions were no more frequent or severe than those that occurred after measles or smallpox vaccination alone, but there was, in this study, a diminished immune response to measles. The last observation was not, however, confirmed in subsequent studies. From these and other observations, Foege and Foster[112] concluded that it was safe and efficacious to administer simultaneously all the vaccines (oral poliovirus, DPT, measles, and BCG) employed in the WHO Expanded Program of Immunization as well as smallpox and yellow fever vaccines.

Complications of Vaccination

Skin Infections. After vaccination, three types of abnormal skin reactions may occur as follows: (1) eczema vaccinatum and (2) progressive vaccinia, which are both associated with abnormal host reactions, and (3) generalized vaccinia. Vaccinia virus from a lesion may also be accidentally inoculated at other sites on the body or transferred to others. The approximate frequency of such complications and rates per million vaccinees are shown in Tables 6–2 and 6–3, based on a national survey by Lane and colleagues[113] in the United States, the only country in which comprehensive studies of this type were undertaken. More detailed prospective studies in 10 states[114] revealed higher rates for eczema vaccinatum, generalized vaccinia, and accidental infection as well as for other complications; the higher rates resulted from the detection of more minor complications.

Eczema vaccinatum occurs in both vaccinated persons and their unvaccinated contacts who have active or quiescent eczema. Either concurrently with or shortly after the development of the local vaccinial lesion or after an incubation period of 5 days in an unvaccinated eczematous contact, a vaccinial eruption occurs at sites that are eczematous or that had previously been so. The areas

Table 6–2. COMPLICATIONS OF SMALLPOX VACCINATION IN THE UNITED STATES, 1968

VACCINATION STATUS AND AGE (yr)	ESTIMATED NUMBER OF VACCINATIONS	NUMBER OF REPORTED CASES (deaths)					
		Postvaccinal Encephalitis	Progressive Vaccinnia	Eczema Vaccinatum	Generalized Vaccinia	Accidental Infection	Other
Primary vaccinations							
<1	614,000	4 (3)	—	5	43	7	10
1–4	2,733,000	6	1	31	47	91	40
5–9	1,553,000	5 (1)	1 (1)	11	20	32	8
10–19	406,000	—	1 (1)	3	5	3	1
≥20	288,000	1	2	7	13	4	5
Unknown		—	—	1	3	5	2
Total	5,594,000	16 (4)	5 (2)	58	131	142	66
Revaccinations							
<1	—	—	—	—	—	—	—
1–4	478,000	—	—	1	—	1	1
5–9	1,643,000	—	1 (1)	4	1	3	2
10–19	2,657,000	—	1	3	—	—	—
≥20	3,796,000	—	4 (1)	—	9	3	6
Total	8,574,000	—	6 (2)	8	10	7	9
Unvaccinated contacts		—	—	60 (1)	2	44	8
Total	14,168,000	16 (4)	11 (4)	126 (1)	143	193	83

From Lane JM, Ruben FL, Neff JM, Millar JD. Complications of smallpox vaccination, 1968. National surveillance in the United States. N Engl J Med 281:1201–1208, 1969.

become intensely inflamed, and the eruption sometimes spreads to normal skin. Constitutional symptoms are usually severe, with high temperature and generalized lymphadenopathy. Treatment with vaccinia immune globulin appears to reduce mortality.[115]

Progressive vaccinia occurs in persons who suffer from deficient immune mechanisms, such as agammaglobulinemia, defective cell-mediated immunity, or immune deficiency associated with tumors of the reticuloendothelial system or the use of immunosuppressive drugs. In such patients, the vaccinia lesion fails to heal; secondary lesions sometimes appear elsewhere on the body and then gradually spread. Methisazone (*N*-methylisatin β-thiosemicarbazone) is reported to be partially effective in treatment,[116] but one third of such patients die.[113]

With *generalized vaccinia*, one to many lesions develop in 6 to 9 days after vaccination at locations other than the vaccination site in otherwise healthy persons. The evolution of these lesions follows the same temporal course as that of the vaccination lesion itself. Although patients may experience high fever and malaise, an uneventful recovery without the need for specific therapy is usual.

Accidental inoculation of vaccinia virus, transferred from the lesion at the vaccination site, is by far the most common, although innocuous, complication. The most common sites for inoculation are the eyelids, vulva, and perineum. Such lesions evolve rapidly and heal at the same time as the primary lesion. Accidental infection of normal contacts may also occur.

Table 6–3. COMPLICATIONS PER 1 MILLION SMALLPOX VACCINATIONS IN THE UNITED STATES DURING 1968

VACCINATION STATUS AND AGE (yr)	POSTVACCINAL ENCEPHALITIS	PROGRESSIVE VACCINIA	ECZEMA VACCINATUM	GENERALIZED VACCINIA	ACCIDENTAL INFECTION	OTHER
Primary vaccination						
1	6.5	—	8.1	70.0	11.4	16.3
1–4	2.2	*	11.3	17.2	33.3	14.6
5–9	3.2	*	7.1	12.9	20.6	5.2
10–19	—	*	*	12.3	*	*
20	*	*	24.3	45.1	13.9	17.4
Total	2.9	0.9	10.4	23.4	25.4	11.8
Revaccination						
1	—	—	—	—	—	—
1–4	—	—	*	—	*	*
5–9	—	*	2.4	*	*	*
10–19	—	*	*	—	—	—
20	—	1.1	—	2.4	*	1.6
Total	—	0.7	0.9	1.2	0.8	1.0

*Fewer than 4 cases; rate not computed.

From Lane JM, Ruben FL, Neff JM, Millar JD. Complications of smallpox vaccination, 1968. National surveillance in the United States. N Engl J Med 281:1201–1208, 1969.

Postvaccinal Encephalopathy and Encephalitis. Among those without known contraindications to vaccination, postvaccinal encephalopathy and encephalitis are the most serious complications. The incidence of these related complications was substantially higher in Europe after the use of strains in common use at that time[117] than in the United States, where the New York City Board of Health strain was employed. Two pathological forms were distinguished by de Vries[118]: encephalopathy primarily in children younger than 2 years, and encephalitis or encephalomyelitis in those who were older. The encephalopathy is characterized by general hyperemia of the brain, lymphocytic infiltration of the meninges, widespread degenerative changes in ganglion cells, and perivascular hemorrhage. Severe symptoms begin abruptly within 6 to 10 days after vaccination,[119] with fever and convulsions, usually followed by hemiplegia and aphasia; death, when it occurs, follows within a few days. Recovery is seldom complete; the patient is left with mental impairment and some degree of paralysis. Postvaccinal encephalitis, characterized by perivenous demyelination and microglial proliferation, primarily afflicts persons older than 2 years and is similar to the form of encephalitis observed after vaccination against rabies or after measles infection. Illness usually begins between 11 and 15 days after vaccination and is accompanied by fever, vomiting, headache, malaise, and anorexia followed by disorientation and drowsiness and sometimes convulsions and coma. Death occurs in 10 to 35% of cases, usually within a week. Some survivors have residual paralysis or mental impairment. Paralysis, when it is present, tends to be of the upper motor neuron type. Among those patients who recover fully, symptoms and signs resolve within 2 weeks.[120–127]

Many reports document the frequency of cases of postvaccinal encephalopathy and encephalitis in Europe and the United States, but comparison of rates is difficult because of differing criteria for diagnosis and variability in the completeness of reporting (Table 6–4). The usual levels of incidence, such as those reported from the Netherlands, Germany, and Austria, were higher than those in the United Kingdom, and these rates in turn were higher than those in the United States.[113, 114, 128] Whatever the criteria and methods, differences between the rates appeared to be real, and this fact caused a number of countries, during the 1960s, to begin using the Lister strain, then in use in the United Kingdom. A dramatic reduction in the incidence of postvaccinal encephalitis subsequently occurred.[120, 129] The incidence in the Netherlands between 1964 and 1971 appeared to approach that in the United States; 10 of the 16 cases were fatal, however, compared with only 4 of 16 cases reported in the United States in 1968. The differences are not statistically significant, but the results

Table 6–4. INCIDENCE OF POSTVACCINAL ENCEPHALOPATHY (IN INFANTS YOUNGER THAN 2 YEARS) AND POSTVACCINAL ENCEPHALOMYELITIS (IN PERSONS OLDER THAN 2 YEARS) AFTER PRIMARY VACCINATION, IN VARIOUS COUNTRIES AND AT VARIOUS TIMES

	ENCEPHALOPATHY (age <2 yr)			ENCEPHALOMYELITIS (age >2 yr)		
COUNTRY AND INVESTIGATOR	Number of Cases	Number of Vaccinations	Cases per Million	Number of Cases	Number of Vaccinations	Cases per Million
Austria 1948–1953 (Berger and Puntigam,[121] 1954)	6	58,438	103	26	21,323	1219
England and Wales 1951–1960 (Conybeare,[122] 1964)	40	2,960,406	14	26	859,963	30
Germany						
Bavaria 1945–1953 (Herrlich,[123] 1954)	51	1,008,000	51	17	140,800	121
Dusseldorf 1948 (Stuart,[124] 1947; Femmer,[125] 1948)	0	28,768	0	14	67,068	209
Hamburg 1939–1958 (Seeleman,[126] 1960)	34	367,390	93	12	26,713	449
Netherlands						
1924–1928 (van den Berg,[127] 1946)	6	155,730	39	127	548,420	232
1940–1943 (Stuart,[124] 1947)	22	441,294	50	56	160,775	348
1959–1963 (Polak,[120] 1973)	34	1,033,000	33	—	—	—
1964–1971 (Polak,[120] 1973)	16	1,495,000	11	—	—	—
United States 1968						
National survey (Lane et al,[113] 1969)	4	614,000*	7	12	4,980,000†	2
10-state survey (Lane et al,[114] 1970)	3	71,000*	42	5	579,000†	9

*Age younger than 1 year.
†Age 1 year or older.
From Fenner F, Henderson DA, Arita I, et al. Smallpox and Its Eradication. Geneva, World Health Organization, 1988, p 307.

are consistent with other observations that suggest that the New York City Board of Health strain is somewhat less pathogenic than the Lister strain.

No single laboratory test correlates with strain virulence, but Marrenikova and colleagues,[130] as a result of a series of studies in mice and rats, provide a broad classification of a number of strains as follows: (1) least pathogenic: New York City Board of Health and EM-63 (a derivative of this strain); (2) moderately pathogenic: Lister, Berne, and Patwadanger (from India); and (3) highly pathogenic, Denmark, Tashkent (an older Russian strain), and Ikeda (an older Japanese strain).

Unusual Complications. In some laboratories, even during the present century, the vaccine was often contaminated with tetanus spores or other pyogenic bacteria that induced infections. With improved methods, however, such infections ceased to occur.

Vaccination during pregnancy did not appear to result in an increase in the incidence of abortions or stillbirths.[131–133] Fetal vaccinia is rare, having been documented on fewer than 20 occasions[134]; no studies have implicated vaccinia virus as a teratogen.[135]

A rare occurrence is the development of a malignant skin tumor, such as a melanoma, in the vaccination scar many years later,[136] and vaccinal osteomyelitis has occasionally been recorded and sometimes confirmed by recovery of vaccinia virus.[137]

Indications for Vaccination

In endemic countries, which, until after World War I, consisted of most of the world, vaccination was recommended for everyone, with revaccination to occur every 3 to 10 years. The only exceptions were infants, for whom primary vaccination was customarily delayed until they were 3 to 12 months of age, mainly because of more frequent vaccination failures at an earlier age. As higher titer vaccines became available in the 1920s, French and then German physicians showed that a high proportion of successful vaccinations could be achieved at birth, and in some hospitals, this became routine practice.[138] In at least one city in the United States, Detroit, neonatal vaccination was mandated in the mid-1920s.[139]

As time passed and smallpox incidence declined, it became increasingly common for smallpox-free countries to delay primary vaccination until children were older. This resulted in part from the demonstration that maternal antibody inhibited virus proliferation[140] and in part from the belief that older children could better cope with the fever and systemic symptoms of vaccinial infection.

Vaccination at a later age was also less likely to be associated mistakenly with other events, such as sudden infant death syndrome, which might be temporally but not causally related. Some European countries recommended that vaccination be delayed until the second year of life to avoid postvaccinal encephalopathy,[119] and the United States adopted the practice of vaccinating at 12 months of age when studies suggested a higher frequency of postvaccinal encephalitis among those vaccinated before 1 year of age than among those vaccinated between 1 and 4 years of age.[113] What these changes in policy may have achieved, however, is unknown, because no studies were performed to validate that complications were subsequently less frequent.

As a rule, most vaccination practices in the developing countries tended to parallel those in Europe and North America, and as of 1967, most countries, even those with endemic smallpox, delayed vaccination until the child was 3 to 9 months of age. Notable exceptions were Hong Kong,[107] where neonatal vaccination had been traditional at least since World War II, and Madras, India,[103] where neonatal vaccination was introduced in the late 1950s. During the late 1960s, it became apparent that vaccines that met international standards of potency consistently resulted in high levels of vaccination "takes" in newborns. Thus, newborn vaccination was recommended for all countries, although not all countries followed the practice. Unfortunately, there are no adequate studies that serve to compare the efficacy and durability of immunity provided at birth with that provided at older ages, nor is there information that permits a comparison to be made between the relative frequency of vaccination complications at this and older ages.

Primary vaccination was provided for adults if required, although many workers have considered it to be associated with a substantially higher incidence of postvaccinal encephalitis and other serious complications. Earlier European data suggest this to be the case,[117] but this was not borne out in studies conducted in the United States.[113, 128] Confirming this association was a review of United States military medical records between 1946 and 1962, conducted by the Centers for Disease Control and Prevention, which revealed no cases of central nervous system complications among an estimated 2 million recruits who were given primary vaccinations. The differences in experience in Europe and the United States almost certainly reflect differences in the pathogenicity of the strains employed.

Since 1980, routine vaccination has ceased in all countries, although a number of countries continue to provide vaccination to military forces as a protection in case variola virus is used as a biological warfare agent. Otherwise, vaccination is recommended only for those working in laboratories where orthopoxviruses are used.

Contraindications to Vaccination

During campaigns in areas that were endemic for smallpox, the WHO recognized no contraindications to vaccination for two reasons: first, the risk associated with smallpox infection was significantly greater than the risk of complications; second, most vaccinations were performed by individuals without medical training who could not be expected to recognize conditions such as eczema or to identify patients with immune deficiency syndromes. It was recommended that only those who were extremely sick not be vaccinated on the grounds that their subsequent death might be attributed mistakenly to vaccination.

In nonendemic areas, four conditions were generally accepted as contraindications.

Immune Disorders. Immune disorders included agammaglobulinemia, hypogammaglobulinemia, neoplasms affecting the reticuloendothelial system, and compromised immune status associated with the use of immunosuppressive drugs. Persons with such disorders, if vaccinated, were at substantial risk of developing the frequently fatal progressive vaccinia.

Eczema. Individuals with active eczema or a past history of eczema were at special risk of developing eczema vaccinatum, a serious and sometimes fatal complication. Because family members with eczema were also at risk from contact spread of vaccinia virus, it was recommended that either the healthy vaccinee or the eczematous family member live apart from the family until the lesion had fully scabbed over.

Pregnancy. Pregnant women were not vaccinated on the general principle that immunization of any sort should be avoided during pregnancy and because of the rare risk of fetal vaccinia.

Disorders of the Central Nervous System. Many countries recognized as contraindications disorders of the central nervous system in potential vaccinees and sometimes their families, hoping, in so doing, to minimize the risk of postvaccinal encephalitis. However, there is no evidence that the exclusion of such persons affected the incidence of that complication.

Some authorities recommended withholding vaccination from patients suffering from various acute or chronic illnesses of many other types, hypothesizing that the response to vaccination might be abnormal. There was no evidence for this occurrence except in the case of leprosy patients, some of whom developed erythema nodosum leprosum or neuritis after primary vaccination.[141, 142] In endemic areas, however, leprosy patients were vaccinated because the risk of smallpox substantially outweighed the risk of complications.

PUBLIC HEALTH

Epidemiological Effects of Vaccination

United States

Smallpox vaccination in the United States began in 1800, but its routine widespread use did not occur until this century. It was first demonstrated by Waterhouse in Boston in July 1800, with material provided by Jenner,[143] and its use was actively promoted by President Thomas Jefferson.[144] Because propagation of the virus at that time was primarily dependent on arm-to-arm transfer of material from a successful vaccinee to others, vaccination was practiced sporadically. Epidemics of variola major continued to occur at intervals, depending on population density and frequency of importations.

Toward the end of the 19th century in the United States, vaccinia virus began to be propagated on the flank of a calf, thus making vaccination more readily and widely available. By 1897, smallpox had largely been eliminated,[3] the result of vaccination and outbreak control. That summer, however, an outbreak of variola minor occurred in Pensacola, Florida, and within 4 years, this variety of smallpox had spread across the country.[145] Although outbreaks of variola major continued to occur until about 1927, most cases of smallpox were caused by variola minor. Because the disease was mild and the case-fatality rate was only 0.3 to 1.0%, interest in vaccination waned. To control the disease, public health authorities sought to compel vaccination as a requirement for school entry, an action upheld by the Supreme Court,[146] a highly effective measure.[147] However, antivaccinationist sentiment and antipathy toward compulsory measures prevailed in many states, most of which passed no legislation or prohibited compulsory vaccination. Reported cases of smallpox declined from 102,791 in 1921 to 30,151 in 1931, and between 1932 and 1939, 5000 to 15,000 cases were reported annually, with 23 to 52 deaths. During the following decade, reported cases steadily diminished, the last occurring in 1949. This progress occurred in the absence of any nationally coordinated smallpox control effort, and little is known about the extent of vaccination immunity in the country during the 1940s or about the epidemiology of smallpox. However, improved smallpox control, and eventually its elimination, is attributed by Leake[148] to the wider availability of better refrigeration and, consequently, better preservation of the vaccine. Routine vaccination continued in the United States until 1971 as a protection in case smallpox was imported and was enforced in most states by compelling vaccination as a requirement for school entry. Beginning in the 1960s, the Centers for Disease Control and Prevention urged the routine vaccination of hospital staff, a group at high risk if smallpox was imported, but few hospitals complied. After the global eradication of smallpox, distribution of vaccine was restricted to the military and to the few laboratories that were working with orthopoxviruses.

Other Industrialized Countries

Through the 1800s, the experience with vaccination in other industrialized countries was similar to that in the United States. After an initial surge of enthusiasm for vaccination in the early 1800s, vaccination was less uniformly and extensively practiced in most countries until near the close of the century, when the vaccinia virus began to be propagated on calves. By 1900, a number of countries in northern Europe became smallpox free, and by 1914, the incidence in most countries had decreased to comparatively low levels. Even so, during the period from 1910 to 1914, Russia experienced a reported 200,000 deaths, and nearly 25,000 deaths were recorded in other European countries.[149, 150] World War I led to a resurgence of smallpox in Russia and its spread from there to many other countries. During the 1920s, vaccination programs led to the interruption of smallpox transmission in many European countries, and by the mid-1930s, smallpox occurred only after importations except in Spain and Portugal. Endemic smallpox persisted in these countries until 1948 and 1953, respectively.

Of the other major industrialized countries, as they are often referred to today, Canada interrupted transmission of smallpox in the early 1940s and Japan about 1950. Vaccination continued in all the industrialized countries, as it did in the United States, until the mid to late 1970s as a protection in case smallpox was reintroduced. Australia and New Zealand were two notable exceptions. These countries, protected by distance and strict quarantine measures, never vaccinated widely but also never became endemic for smallpox.

Eradication from the World

The first commitment to smallpox eradication as such was made in 1950 by the Pan American Sanitary Organization, which decided that year on a hemisphere-wide effort.[151] Freeze-dried vaccine produced by an improved commercial process[39] was employed in mass vaccination campaigns, which during the succeeding decade eliminated smallpox from all countries except Argentina, Brazil, Colombia, and Ecuador.

In 1958, the Soviet Union proposed to the World Health Assembly that the WHO undertake a global eradication program,[32] a proposal that was agreed on in 1959.[33] During the succeeding 7 years, a number of countries embarked on mass vaccination campaigns, and several countries, including China, were successful in eliminating the disease (Fig. 6–6). Overall, however, progress was disappointing, especially in Africa and in the Indian subcontinent. Few countries voluntarily contributed resources, and the WHO, then preoccupied with a costly and disappointing global malaria eradication program, provided few of its own resources and little support.

Frustrated by lack of progress in the program, although skeptical about the feasibility of the concept of eradication itself, the World Health Assembly in 1966 decided, finally, to provide to the WHO a special allocation of $2.4 million annually for an intensified global smallpox eradication effort.[34] The hope was expressed that the task might be accomplished within a 10-year period, that is, by December 1976.[36]

In the intensified program, the strategy emphasized two principles that ultimately proved to be critical to its success. The first was to ensure, through the use of international vaccine testing centers, that all vaccine in the program met accepted standards and, likewise, to ensure, through concurrent sample surveys, that a satisfactory vaccination coverage had been achieved and that the vaccinations had been successful. The second principle was the identification of the absence of cases as the program's principal objective and the need to measure progress not in terms of numbers of vaccinations performed, as had been the practice, but in terms of declining incidence of smallpox. This principle required the development of an effective case notification system and focused attention on measures to reduce incidence.

During 1967, the first year of the program, 44 countries, 31 of which were endemic, reported 131,789 cases of smallpox. The endemic countries were Brazil, most countries of Africa south of the Sahara, and five countries in Asia: Afghanistan, India, Indonesia, Nepal, and Pakistan (see Fig. 6–6). Later surveys revealed that only about 1% of all cases were then being reported; thus, it is estimated that between 10 and 15 million cases occurred that year in countries whose population was about 1.2 billion people.

Provision of adequate supplies of fully potent vaccine was a critical first problem.[152, 153] Early surveys revealed that not more than 10% of the vaccine being produced in or provided to the endemic countries met accepted

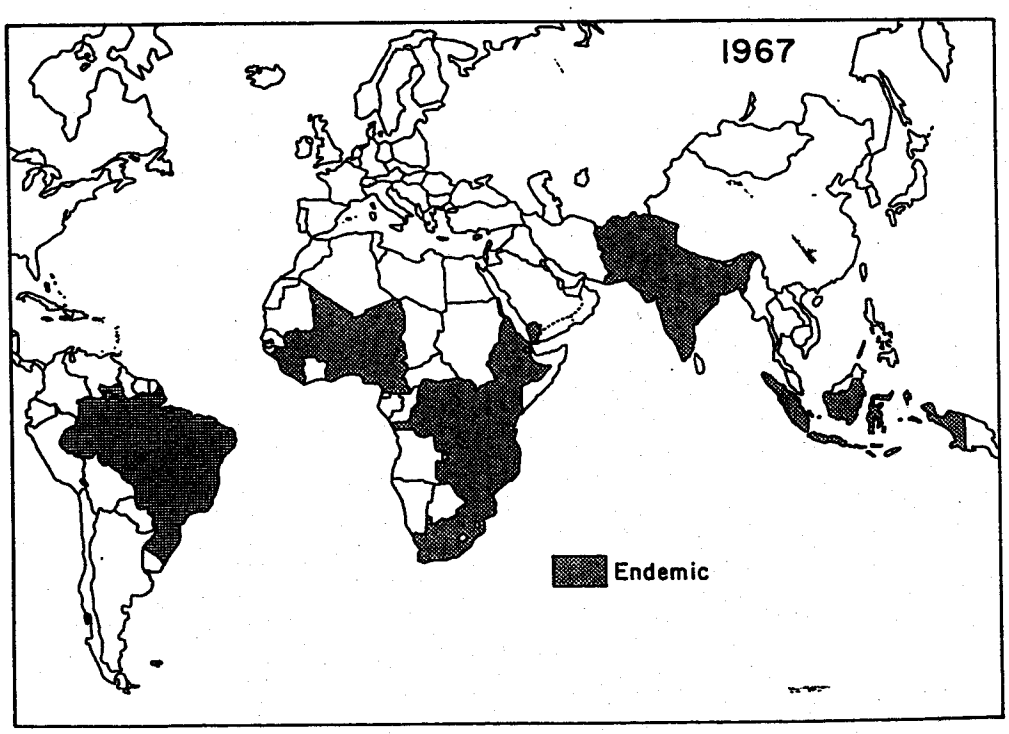

Figure 6–6. Countries with endemic smallpox in 1967 when the intensified program was initiated.

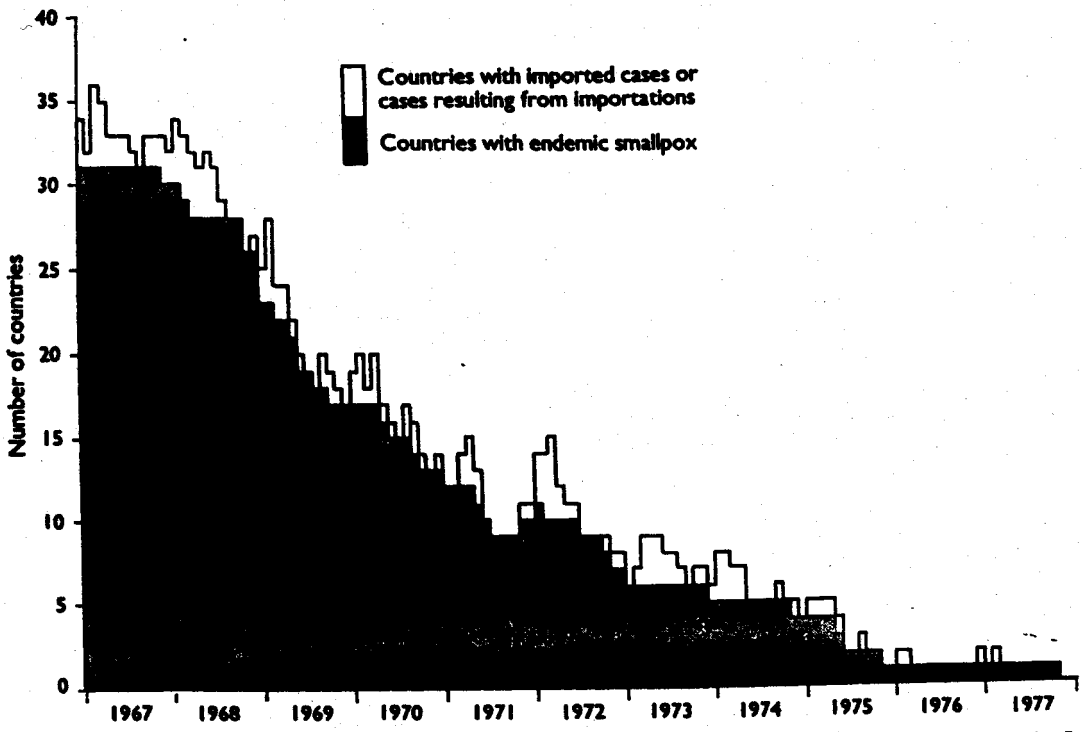

Figure 6–7. Number of countries experiencing smallpox each year from 1967 to 1978. (From Fenner F, Henderson DA, Arita I, et al. Smallpox and Its Eradication. Geneva, World Health Organization, 1988, pp 517–538.)

international standards. Laboratories in Canada and the Netherlands agreed to test samples of all vaccine to be used in the program, manufacturers collaborated in developing a detailed production manual, and consultants and equipment were provided to laboratories in the endemic countries. Donations of vaccine, primarily from the Soviet Union and the United States, met initial needs, but by 1973, more than 80% of all vaccine for the program was being produced in the developing countries.

The traditional method of vaccination by scarification was changed. In 1967, jet injectors were introduced for programs in Brazil and western and central Africa. One year later, a new instrument, the bifurcated needle, developed by Wyeth Laboratories, was found to be effective in multiple-puncture vaccinations[154]; by 1969, it was in use in all countries. Vaccination with the bifurcated needle required only one fourth as much vaccine, even illiterate village volunteers required less than an hour's training in its proper use, and workers could vaccinate as many as 1000 persons per day.

Vaccination programs were developed or strengthened in all endemic and neighboring countries, the last of them beginning in 1971. Although the strategy also called for the improvement of national reporting systems and containment of outbreaks by special teams, such activities were slow to begin. It quickly became apparent, however, that these activities, referred to as the surveillance-containment program, could serve to interrupt smallpox transmission more easily and quickly than anyone had imagined, even where vaccinial immunity was low.[69, 155, 156]

With increasingly greater emphasis on surveillance-containment activities, endemic smallpox steadily receded (Fig. 6–7; see also Fig. 6–6). It was eliminated from 20 countries of western and central Africa by 1970,[70] from Brazil in 1971, from Indonesia in 1972, and from the entire continent of Asia in 1975. Ethiopia stopped transmission in 1976 and Somalia on October 26, 1977. The last naturally occurring case of smallpox developed less than 1 year after the originally projected 10-year target date. WHO-organized international commissions visited each of the endemic countries and areas to confirm the fact of eradication, and in May 1980 the World Health Assembly, acting on the recommendation of a WHO Global Commission (Fig. 6–8), announced that worldwide eradication had been achieved and recommended that smallpox vaccination be used only for those working with orthopoxvirus in research laboratories.[1] The WHO established an international stockpile of vaccine in the unlikely event that its use would ever again be required and encouraged laboratories to destroy their stocks of variola virus. As of 1997, variola virus remained in only two research laboratories—one in the United States and one in Russia.

The overall cost of the program was estimated to be about $300 million, of which $98 million represented international assistance. The savings, as a result of cessation of vaccination and quarantine measures, was estimated to be in excess of $1 billion annually.[4]

With the eradication of smallpox, questions arose as to whether it might not be prudent to destroy the known remaining laboratory stocks of variola virus to provide added assurance that the virus might not accidentally or even deliberately be released into an unprotected world. This was considered in 1986 by a WHO Ad Hoc Committee on Orthopoxvirus Infections, which recommended a broader consultation with the international

Figure 6–8. Document signed on December 9, 1979, by members of the World Health Organization Global Commission, certifying that smallpox had been eradicated. (From Fenner F, Henderson DA, Arita I, et al. Smallpox and Its Eradication. Geneva, World Health Organization, 1988, frontispiece.)

community and destruction of the virus if no serious objections were raised.[157] Meanwhile, in preparation for possible destruction, a library of cloned DNA restriction fragments of selected strains was prepared, and later the genomes of a number of prototype strains were fully or partially sequenced.[158]

Arguments were advanced both supporting[158] and objecting to[159] destruction of the virus stocks. In 1994, the question was again reviewed in depth by the WHO Committee, which again recommended to the WHO Director General that the considerations, on balance, strongly favored destruction of the virus.[160] It was ultimately decided in the 1996 World Health Assembly that destruction of the virus should take place on June 30, 1999.

RECOMBINANT VACCINIA VIRUS VACCINES

Shortly after the World Health Assembly resolution recommending cessation of smallpox vaccination, proposals were made to use recombinant vaccinia viruses for immunization against other infectious agents.[161, 162] The idea was to stably insert one or more genes of other pathogens into the genome of vaccinia virus while retaining the infectivity of the latter. Moreover, the large capacity of vaccinia virus for foreign DNA raised the possibility of polyvalent vaccines against multiple diseases.[163, 164] In principle, recombinant vaccinia viruses would have many of the properties of live attenuated virus vaccines and would present antigens in natural ways so as to stimulate humoral immunity to native protein conformation as well as cell-mediated immunity. Such vaccines might also retain the familiar advantages of smallpox vaccine: heat stability, low cost, ease of administration, and a scar as visible proof of vaccination. Although recombinant vaccinia viruses are still undergoing investigation for human and veterinary vaccination, their great value for vaccine research has been widely recognized.[165]

Construction of Recombinant Vaccinia Virus Vectors

The development of recombinant vaccinia viruses depended on a method of introducing a foreign gene into the vaccinia virus genome. Homologous recombination between poxviruses was well known and had been demonstrated by coinfecting cells with two viruses[166] and by infecting cells with one virus and transfecting them with genomic DNA[167, 168] or cloned DNA segments.[169] It is likely that DNA recombination occurs in the cytoplasm by enzymes encoded by vaccinia virus. Less well understood at the time was how to achieve expression of foreign genes. The recognition of vaccinia virus promoter elements provided a general method of preparing vaccinia virus expression vectors[161, 170, 171] that is illustrated in Figure 6–9. Insights achieved through basic studies of vaccinia virus promoters have led to substantial improvements in the level of gene expression.[172, 173] Other innovations, including alternative methods of selecting or identifying recombinant vaccinia viruses and the insertion of foreign genes by direct ligation, are summarized elsewhere.[174]

Selection of a Vaccinia Virus Strain

The WR strain of vaccinia virus, favored for basic poxvirus research in the United States and widely used to make recombinant viruses for laboratory studies, is unsuitable for vaccines. The four vaccinia virus strains administered most often for smallpox vaccination were EM-63, Lister, New York City Board of Health, and Temple of Heaven. Of these, the New York City Board of Health strain had relatively low pathogenicity[175] and was chosen to make a recombinant vaccinia virus intended for human use. Although the latter appeared to be safe in a small clinical trial,[176] further attenuation of recombinant vaccinia viruses seems prudent for large-scale administration. Several approaches have been taken to achieve a safer vector.

Although 50 or more of the nearly 200 genes of vaccinia virus are dispensable for replication in tissue culture cells, the deletion of some of these genes reduces virulence in animal models.[177–179] The deletional approach to making a safe vector was exemplified by the removal of 18 genes from the Copenhagen strain of vaccinia virus, thereby producing a highly attenuated derivative called NYVAC.[180] Several studies have indicated that NYVAC has good potential for human and veterinary vaccines.[181, 182] An alternative approach was to use one of several highly attenuated strains of vaccinia virus that were derived by serial passages in vitro and

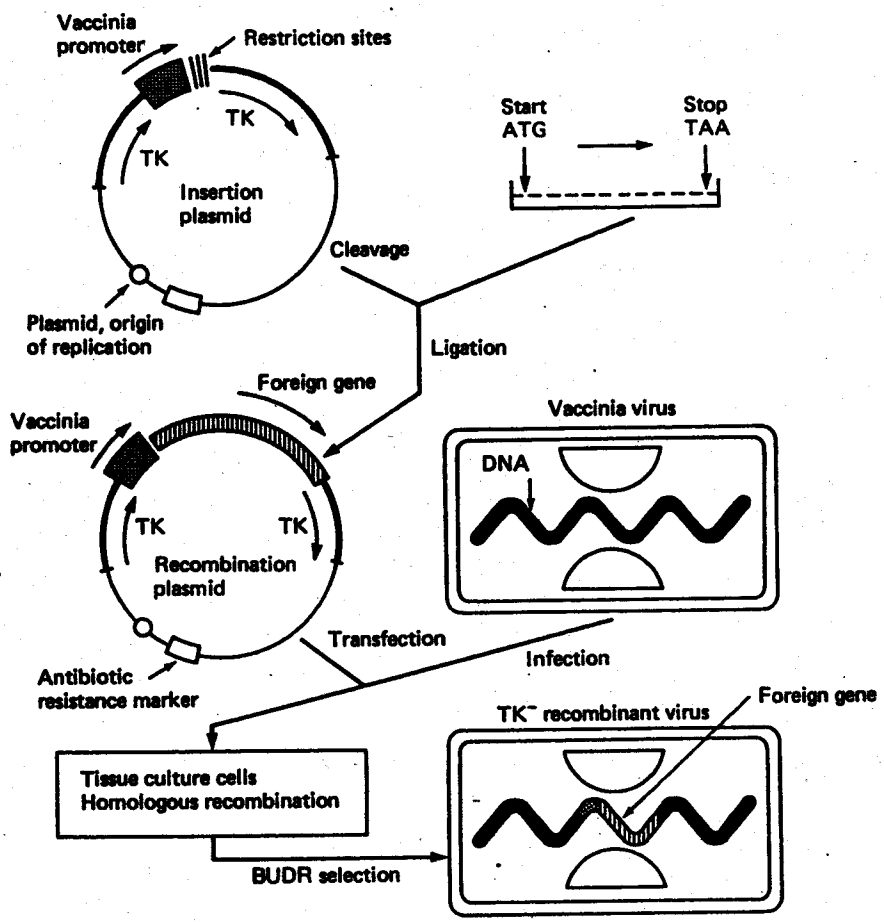

Figure 6–8. The insertion plasmid (or transfer vector) contains restriction endonuclease sites for ligation of the complete open reading frame (ATG----------TAA) of a foreign gene adjacent to a vaccinia virus promoter as well as flanking vaccinia virus DNA sequences (in this case from the thymidine kinase [TK] gene) to direct homologous recombinational insertion into the vaccinia virus genome. Tissue culture cells are infected with vaccinia virus and then transfected with the insertion plasmid, resulting in the formation of a stable recombinant virus. Because only about 0.1% of the progeny are recombinant viruses, selection techniques are frequently used. In the example shown here, the recombinant virus has an interrupted TK gene and can be selected by use of 5-bromodeoxyuridine (BuDR). Detailed protocols for constructing recombinant vaccinia viruses are available.[171]

had been tested in humans before the eradication of smallpox.[175] One of these strains, MVA, suffered multiple deletions and became severely host range restricted during more than 500 passages in chick embryo cells, providing a high degree of attenuation.[183, 184] Laboratory studies demonstrated unimpaired MVA gene expression in human cells and a block in virion morphogenesis.[185] The ability to achieve high expression of recombinant genes despite abortive replication is a remarkable feature of this mutant virus. Even though MVA probably does not replicate significantly in animal models, excellent immune responses to recombinant proteins were obtained; moreover, the dose required was similar to that of a standard replication-competent strain.[186–188]

Vaccinia Virus as a Tool for Vaccine Research

Recombinant vaccinia viruses provide a powerful means of dissecting the immune responses of humans and experimental animals to individual gene products of infectious agents. Only a few examples can be mentioned here. Thus, recombinant vaccinia viruses were used to demonstrate that the HA and NP proteins of influenza virus induced subtype-specific and cross-reactive cytotoxic T-cell responses, respectively.[189, 190] Evidence of human immunodeficiency virus type 1 (HIV-1)–specific cytotoxic T cells in patients with acquired immunodeficiency syndrome (AIDS) was first obtained by use of recombinant vaccinia viruses expressing the envelope or internal proteins to prepare target cells.[191, 192] Indeed, recombinant vaccinia viruses have become an important tool for cellular immunologists.[193] Because proteins expressed in mammalian cells by recombinant vaccinia viruses are folded, processed, and transported normally, they can be used to either induce or bind antibodies that recognize conformational epitopes.[194]

The wide host range of vaccinia virus makes it possible to determine protective immune responses against infectious agents in a variety of experimental animals from rodents to primates. For example, the F glycoprotein is most important for inducing protection to respiratory syncytial virus,[195] whereas the HN protein is better for parainfluenza virus type 3[196] and type 5.[197] Protection elicited by the respiratory syncytial virus M2 protein is due to $CD8^+$ T cells, whereas that induced by the F and G proteins is due to antibodies.[198] Similar results, with respect to the HA and NP proteins, have been obtained in studies with influenza virus.[199] In some cases, vaccination has a priming effect that is followed by an anamnestic antibody response, as indicated for the protection of chimpanzees after inoculation with a recombinant vaccinia virus expressing the hepatitis B surface antigen.[200] A list of viruses for which protective immune responses have been obtained may be found in a review.[201]

The induction of strong cytotoxic T-cell responses

elicited by recombinant vaccinia viruses has led to their evaluation as tumor vaccines in model systems.[202-205]

Other Poxvirus Vectors

The procedures developed for the construction of recombinant vaccinia viruses have been applied to members of other poxvirus genera including avian poxviruses[206] and capripoxviruses.[207] Although the avian poxviruses are naturally host range restricted, gene expression and protective immunity can be established in nonavian species.[208, 209] As nonreplicating vectors, avian poxviruses should be exceptionally safe recombinant vaccines.

Human Vaccines

Although vaccinia virus vectors have proved extremely useful for vaccine research as well as for research in many other fields, the potential for human vaccines is still under investigation. As for all vaccines, the critical factors include safety and efficacy as well as the facility for vaccine production, distribution, and administration. In addition, there are special questions regarding prior immunity to vaccinia virus acquired either through smallpox vaccination or through a recombinant vaccine and the design of polyvalent vaccines.

Safety issues have been minimized by the demonstration that host-restricted or "nonreplicating" vaccinia virus vectors, such as the MVA and NYVAC, are immunogenic. At the National Institutes of Health intramural laboratories in Bethesda, research with both of these strains is permitted at biosafety level 1 conditions, whereas level 2 and a recent smallpox vaccination are required for working with standard vaccinia virus strains.

Generic and specific factors are involved in vaccine efficacy. With regard to the former, great improvements in gene expression have been made so that present generation vectors produce many times more recombinant protein than the original vectors. In some instances, immunogenicity has been improved by altering the presentation of the recombinant protein so that it is plasma membrane associated rather than intracellular or secreted.[210, 211] Promising results have been obtained by constructing recombinant vaccinia viruses that coexpress an immunogen and an immunomodulatory cytokine.[212-214]

Although the use of live attenuated viruses as human vaccines may require no special knowledge regarding the targets of immunity, such specific information is needed for recombinant vectors. For many viruses, the membrane glycoproteins or capsids are targets of neutralizing antibody and the internal proteins provide good targets for cytotoxic T cells. Animal models may be helpful in identifying those targets that provide protective immunity. In addition, some infectious agents have special vaccine requirements such as those related to portal of entry, site of replication, antigenic variation, type of immune response needed, and presence of maternal antibodies, which may or may not be met by a recombinant vaccine.

The smallpox vaccine was most frequently prepared from vaccinia virus that was propagated in the skin of an animal, but an approved, stable, freeze-dried vaccine was produced in monolayer cultures of primary rabbit kidney cells.[215] Acceptable cultured cell lines or primary chick embryo fibroblasts would be alternative substrates for virus propagation. Thus, there should be no impediment to the preparation of vaccines that meet present standards of purity. Presumably, procedures for freeze-drying could be adapted to the production of recombinant vaccinia viruses, and it is hoped that such preparations would retain the excellent thermal stability that made a cold chain unnecessary for the smallpox vaccine.

The smallpox vaccine was generally administered by scarification of the skin or less commonly by a high-pressure jet injector.[175] The intradermal route was used for clinical testing of a recombinant virus made with the New York City Board of Health strain.[176] However, other routes (e.g., nasal, oral, subcutaneous, or intramuscular) may be preferred for nonreplicating strains of vaccinia virus.

Although it was based on a small sampling, prior smallpox vaccination appeared to diminish the immune response to a recombinant vaccine.[176] For children and the majority of individuals born during the past 20 years, who have not received a smallpox vaccination, this would not be a problem. However, a poxvirus may not be useful as a carrier for revaccination with a second gene because of the immune response to the vector. Whether prior immunity could be overcome by using vectors that express more recombinant protein or through alternative routes of administration is uncertain. Nevertheless, immunization with a single vector expressing multiple genes, simultaneously with a cocktail of vectors, or successively with distantly related poxvirus vectors might obviate such problems.

Accelerated efforts to develop an AIDS vaccine have led to the human testing of a first-generation recombinant vaccinia virus expressing the HIV-1 envelope gene.[176, 216] The modest immune response detected may be due in part to the relatively weak promoter used and the failure to eliminate poxvirus early transcriptional stop signals within the HIV-1 gene.[217] The HIV-1 neutralizing antibody response, however, was augmented by secondary immunization with a subunit HIV-1 envelope protein.[218, 219] There is considerable enthusiasm for such a prime-boost strategy because it can stimulate cell-mediated and humoral immunity. Prime-boost vaccinations carried out with a recombinant canarypox virus and recombinant HIV-1 envelope protein induced HIV-specific cytotoxic cells and neutralizing antibody in phase I clinical trials. Expanded phase II trials are in progress.[220-222]

A recombinant vaccinia virus that expresses the major Epstein-Barr virus membrane glycoprotein was immunogenic when it was administered to infants and young children and may have delayed or prevented natural infection for a period of 16 months.[223] A recombinant canarypox virus expressing the rabies virus glycoprotein was safe in humans, induced functional antibody to rabies glycoprotein, elicited cellular responses to rabies virus, and could be used successfully for boosting at a 6-month interval.[224, 225]

Veterinary and Wildlife Vaccines

Substantially different factors are involved in the applicability of vaccines for medical and veterinary practices.[226] Economic criteria, although of considerable importance for human vaccines in developing countries, are decisive for most veterinary vaccines. Also, there is far more latitude in the manufacture and use of veterinary vaccines than has been permitted by regulatory agencies for human vaccines. In addition, durable immunity is not important for livestock, and a small number of vaccine-associated illnesses can be tolerated in veterinary vaccines. Because live fowlpox virus vaccines are already used in the poultry industry to prevent fowlpox, recombinant poxvirus vaccines should be practical.

Recombinant vaccinia viruses have been shown to protect animals against diseases of veterinary importance including vesicular stomatitis virus[227] and rinderpest[228] in cattle, pseudorabies virus in swine,[229] and influenza virus in chickens.[230] A recombinant vaccinia virus expressing the rabies virus glycoprotein[231] has been successfully administered in bait form as a wildlife vaccine in both the United States and Europe.[232, 233]

Other poxviruses are also being tested as vectors for veterinary applications. Examples include a raccoon poxvirus vector for raccoons against rabies virus[234]; a capripoxvirus vector for cattle against rinderpest virus[235]; a swinepox virus vector for pigs against pseudorabies virus[236]; fowlpox vectors for chickens against influenza virus,[237] Newcastle disease virus,[238, 239] and infectious bursal disease virus[240]; and canarypox virus for cats against feline leukemia virus.[241]

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